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Analysis of Protein Content, Spectrophotometry FT-IR, and Antibacterial Effects of Earthworm (*Eudriluseugenia*)

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Abstract

This research to compare effectiveness of coelomic fluid and extract ethanol of *Eudriluseugeniae* against *Staphylococcus aureus* and *Escherichia coli* as antibacterial. Suggest that protein on *Eudriluseugeniae* have antibacterial activity.Experimental laboratory with analytical statistic Mann- Whitney with following step of research: (1) Determination of earthworm, (2) Cold shock methods modification to get coelomic fluid, (3) Maceration ethanol 70 % of *Eudriluseugeniae*.(4) Determination protein content with Kjehdahl methods, (5) Identification absorbance infrared by spectrophotometry FT-IR .(7) Antibacterial assay using disc diffusion against *Staphylococcus aureus* and *Escherichia coli*.The result of this study showed that protein content of coelomic fluid (1.875 %)were higher than ethanol extract (0.437%) of *Eudrilus eugenia*. Coelomic liquid had no functional group in the chemical compound. However, the ethanol extract showed some functional group includes aliphatic, ethanol, and Amide A group. The antibacterial effect against *Escherichia coli* and *Staphylococcus aureus* from ethanol extract or coelomic liquid of *Eudriluseugeniae* were similar at equal concentration. It due to p value of analysis was lower than 0.05.Coelomic liquid and ethanol extract had similar antibacterial effects against *Staphylococcus aureus* and *Escherichia coli*.

Keywords: *Eudriluseugeniae*; coelomic fluid; Ciprofloxacin; *Staphylococcus aureus* and *Escherichia coli*.

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1. Introduction

Topical, oral, and injection antibiotic widely used and uncontrolled, which caused resistant of bacteria through the drug, impairment of normal flora, increased morbidity, cost, and period of antibiotic usage. Sri SelviaNingsih, N.K., et.al. (2016) and Chudlori, B. and his colleagues (2012) reported *Staphylococcus aureus*were more sensitive against imipenam than ciprofloxacin, while *Escherichia coli* as negative gram bacteria which often found in most of Pus in Regional Public Hospital of dr. Moewardi 75% were resistant against ciprofloxacin. These defensive responds were caused by acquisition and transfer of resistant plasmid which was part of intrinsic resistant mechanism. It was found especially in flourquinolon such as ciprofloxacin, some type of beta lactam such as penicillin, and tetracycline. Other study show that there were plasmid gene and chromosome mutation in gyrA and parC gene on Escherichia coli which were resistant through many type of antibiotic that usually used in Urinary tract infection therapy[1–4].

Earthworm have been know as food and traditional drug material, which has some health benefits [5,6], such treat fever, anaesthetic, detoxification, treat hypertension, joint inflammation and itch [7]. These health benefits due to enzymes and peptide which are have antibacterial, antioxidant, and anti-inflammatory activity [8].

Coelomic liquid and ethanol extract of Eudriluseugenia has anti bacteria and antifungal effect due to amount of 40 protein, nitrogen, lipid and peptide enzyme (lysosim and fetidin). They are act as humoral and cellular immunity which responsible for cytolytic, proteolytic, antimicroba, hemolitic, and mitogenic[9,10].

Based on the information above, this study aimed to investigate antibacteria effect of Eudriluseugenia for Staphylococcus aureus as gram positive bacteria and Escherichia coli as gram negative bacteria.

2. Methods

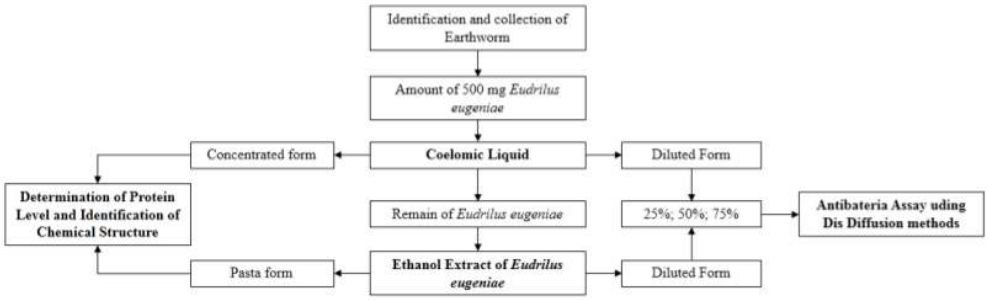

Figure 1: Research Procedure

2.1. Materials

Eudrilus Eugenia, Isolated colonies of *Staphylococcus aureus* and *Eschericia coli,* plastic wrap, Ethanol 70%, aquadest, cotton swab, cotten, Nutrien broth, MHA (Muller Hiton Agar), Alcohol 96%, syringe 1 cc, Acceptible

Disc Ciprofloxacin 5 μg OXOID.

2.2. Colletion of Samples

Eudriluseugenia (African Earthworm)were gotten from livestock in JalanSagu XI Medan Tuntungan which were chosen health and matur earthworm.

2.3. Preparation of Samples

Amount of 500 g *Eudriluseugenia* which had been cleaned and washed by aquedest was put in to funnel glass using filter paper. The funnel glass was directed in to 500 ml Erlenmeyer, and top of the funnel glass was cover by plastic wrap. These was saved in refrigerator for 4-5 hours, after that it was gotten coelomic liquid of *Eudriluseugeniae*.

The remain of *Eudriluseugeniae* was put in to 500 ml beaker glass and dried by *Blast Air Oven Yenaco* YNCOV 53L at 60^{o}C for 4 days, followed by stirring twice in a day using stirrer bar. After that it was gotten 73.01 gram of dry extracts. The dry extract was macerated using 250 ml 70% ethanol followed by stirring until it was homogen in the dark jar which had been filled by silica gel for 3 days. Furthermore, it was filter using funnel glass and filter paper twice, and the ethanol extract was the last filtrate, the residue of filtration was macerated again using 125 ml 70% ethanol.

In the purpose of determination of Protein content and Chemical Structure by Spectrophotometry FT-IR, ethanol extract of *Eudriluseugenia* was form in to pasta using Water Bath Constant Temperature at 75^{o}C for 4 days. After that, each 0.05 gram of pasta form was diluted twice using aquadest and methanol (1:1)

On the other hand, coelomic liquid and ethanol extract of *Eudriluseugeniae* was diluted using aquabidest for antibacterial assay. The dilution was result various concentration of coelomic and ethanol extract includes 25%, 50%, and 75%.

2.4. Determination of Protein Content

Amount of 2.5 ml coelomic liquid and diluted ethanol extract was identified by kjehdahl distillation methods. Amount of protein content were express in percent (%).

2.5. Identification of Chemical Structure using Spectrophotometry FT-IR

Amount of 2.5 ml coelomic liquid and diluted ethanol extract from the earth worm were identified by Spectrophotometry FT-IR.

2.6. Preparation of Media

There were two types of media which are used in this study included nutrion broth and Muller Hinton Agar (MHA). Nutrient broth was made by mixing 0.4 gram powder of nutrient broth into 50 ml aquabidest, while

MHA was made by mixing 17 gram of MHA powder into 500 ml aqubidest. Each of media was stirred by hotplate stirrer untill the media became homogen and then they were autoclave for 15 minutes at 121°C.

2.7. Preparation of Bacteria Culture Specimen

Each colony of bacteria from the pure isolated bacteria of *Staphylococcus aureus* and *Escherichia coli* was put by needle ose and culture into Nutrient broth which had been filled into test tube and incubated for 24 hours at 37°C.

2.8. Antibacterial Assay using Disc Diffusion Methods

The bacteria which had been cultured in Nutrient broth was piped by micropipet into petridish. After that MHA was filled into the petridish and mixed it. At the same time, each concentration of ethanol extract and coelomic liquid which had been diluted was piped in to disc difusion. For the last, each of disc diffusion was put into MHA in petridish, and they were incubated in 37°C for 3 days. After 3 days, the diameter of zone inhibition (clear zone) in MHA was measured using vernier calliper in milimeter (mm).

2.9. Data analysis

All data from diameter of zone inhibition was expressed as Median (Range) for each concentration of sample. While Amount of protein content was expressed in percent. However the result of Analysis of Spectrophotometry FT-IR was expressed as description in a table. Furthermore diameter of zone inhibition at equal concentration between coelomic liquid and ethanol extract was analysed using Mann-whitney Test which P value less than 0.05 were considered statistically significant.

3. Result and Discussion

3.1. Identification of Earthworm (Eudriluseugeniae)

Earthworm was identified in LaboratoriumSistematikaHewanDepartemenBiologiFakultasMatematikadanIlmuAlam (No. Letter: 038/UN5.2.1.11/KRK/2019) at 9 Januari 2019. The result of the identification was shown in the table 1 and table 2 below.

Table 1: Identification from Specific Properties of *Eudriluseugeniae*

No.	Species	Specific Properties
1.	*Eudriluseugeniae*	Length of body was 10 cm. Shape of body was flat and tip tailed. Head to clitellum distance was 3-4 cm. The body had 135-350 of segments and had clitellum at 30th-32nd segments. It has metameres which are group of all body segments. There were two reproduction organ (hermaphrodite). There were not skleton but had coelomic liquid which were act as hydrostatic frame. The body has reddish-brown colored.

Table 2: Taxonomy of *Eudriluseugeniae*

Kingdom	*Animalia*
Phylum	*Annelida*
Class	*Clitellata*
Ordo	*Haplotaxida*
Family	*Eudrilidae*
Genus	*Eudrilus*
Species	*Eudriluseugeniae*

Eudriluseugeniae or african earthworm has high content of protein [5]. Ethanol extract and coelomic fluid of *Eudiluseugeniae* has antibacterial and antifungal effect as nature product content [8,9]. However, based on Chauhan and his colleagues (2014) reported that extract of *Eudriluseugenia* does not have antibacteria effect against *Eschericia coli* and any types of fungal[10].

3.2. Determination of Protein Content

Determination of protein content from the coelomic liquid and ethanol extract in percent are shown in table 3 below.

Table 3: Amount of Protein Content in Coelomic liquid and Ethanol Extract *Eudriluseugeniae*

No	Sample	Level of Protein
1.	Coelomic fluid	1.875
2.	Ethanol extract	0.437

Based on table 3 above, amount of protein content in coelomic liquid is higher than ethanol extract. Hrenzjak and his colleagues (1991) reported same result that there was higher protein content in the coelomic liquid due to glycoprotein G90. Other protein might be found in earthworm which had antibacterial effect. In *Pheretimajavanica,* there wereprotein which had molecular weight between 7.0 kDa – 55kDa. These proteins usually had tryptophan as monomer (amino acid) [11,12].

3.3. Identification of Chemical Structure Using Spectrophotometry FT-IR

The results of identification of chemical structure using spectrophotometry FT-IR are shown in table 4 below.

Table 4: The result of Spectrophotometry FTIR from Coelomic Liquid and Ethanol Extract *Eudriluseugeniae*

No.	Sample	Absorption Bands	Peak	Type of Bond	Conclusion
1.	Coelomic Liquid	None or Minimal	-	-	-
2. \	Ethanol extract	3300-3311 cm-1	Moderate	Stretching Vibration	Ethanol group
		2925.8 cm^{-1}	Moderate	Stretching and Bending Vibration ($-NH_2$)	Amide A Group (Primer)
		2885.248 cm^{-1}	Moderate	Stretching and Bending Vibration CH	Aliphatic group
		1606.77 cm^{-1}	Moderate	Stretching Vibration (C=C)	Alipatic system group
		1442.75 – 1398.78 cm^{-1}	Moderate	Stretching Vibration ($-CH_3$)	
		1099.43-1025.64 cm^{-1}	strong	Stretching Vibration (C-O-C) antisymmetric	

Based on the table 4 above, coelomic liquid had none or minimal absorbance which meant there no functional group in the chemical compound. However, the ethanol extract showed some functional group includes aliphatic, ethanol, and Amide A group. Amide A group showed presence of amino acid as monomer of Protein. The result of Spectrophotometry showed as oppose to result of determination protein contents. Method which was used to determination of protein content had some disadvantages. Some compound which are not protein also analyse such as purine, pyrimidine, vitamin, large amino acid, or creatinine [13].

3.4. Antibacterial Assay using Disc Diffusion

The result of Mann-whitney test for Diameter of Zone Inhibition from coelomic liquid and ethanol extract was shown in table 5 and 6 below.

Table 5: Comparison of Antibacterial Effect of Ethanol Extract and Coelomic Liquid from *Eudriluseugenia* against *Escherichia coli*

Concentration	Diameter of Zone Inhibition		P Value
	Ethanol Extract [Median (R)]	Coelomic Liquid [Median (R)]	
25%	0 (0)	6.6 (0)	0.083
50%	7.18 (0.15)	6.85 (0.1)	0.121
75%	9.25 (0.70)	7.2 (0.2)	0.121
Positive control	19.58 (2.58)		-
Negative Control	0 (0)		-

Table 6: Comparison of Antibacteria Effect of Ethanol Extract and Coelomic Liquid from *Eudriluseugenia* against *Staphylococcus aureus*

Concentration	Diameter of Zone Inhibition		P Value
	Ethanol Extract[Median (R)]	Coelomic Liquid[Median (R)]	
25%	6.7(0.20)	8 (0.2)	0.121
50%	7.15 (0.10)	10.73 (1.55)	0.121
75%	7.45 (0.10)	13.75 (2.7)	0.121
Positive control	25.53 (1.22)		-
Negative Control	0 (0)		-

Based on table 5 and 6 above, the antibacterial effect against *Escherichia coli* and *Staphylococcus aureus* from ethanol extract or coelomic liquid of *Eudriluseugenia* were similar at equal concentration. It due to p value of analysis was lower than 0.05.

According to Greenwood, Slack, and Peutherer (2002) classification which are shown in table 7 below. Antibacterial activity of ethanol extract at various concentration were weak against *Escherichia coli* or *Staphylococcus aureus*. While coelomic liquid had weak antibacterial activity against *Escherichia coli* but had sufficient antibacterial activity at higher concentration against *Staphylococcus aureus*.

Table 7: Classification of Greenwood, Slack, and Peutherer (2002)

Diameter of Zone Inhibition	Inhibition of Growth (Antibacterial activity)
> 20 mm	Very Potent
16-20 mm	Potent
10-15 mm	Sufficient
< 10 mm	Weak

4. Conclusion

Protein content of coelomic liquid were higher than ethanol extract of *Eudriluseugenia*. Chemical compounds which were contained in ethanol extract of *Eudriluseugenia* had ethanol, aliphatic, and amide A compound. While antibacterial effect of coelomic liquid and ethanol extract were similar against *Staphylococcus aureus* and *Escherichia coli*.

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